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16. ABSTRACT

Newly hatched individuals of four estuarine species were exposed to pentachlorophenol (PCP), sodium pentachlorophenate (Na-PCP), or Dowicide® G (79%Na-PCP), in static toxicity tests.

The 96-hour LC_{50} values for sheepshead minnow (Cyprinodon variegatus) fry exposed to PCP at ages 1-day, 2-week, 4-week, and 6-week were 329, 392, 240, and 223 µg/ℓ, respectively. The 96-hour LC_{50} value for 2-week-old fry exposed to Dowicide® G was 516 µg/ℓ. The larvae (48-hour post hatch) of pinfish, Lagodon rhomboides, were particularly sensitive to Na-PCP (96-hour LC_{50}:38 µg/ℓ) and Dowicide® G (96-hour LC_{50}:66 µg/ℓ). For 24-hour-old grass shrimp (Palaemonetes pugio) larve exposed to NA-PCP the 96-hour LC_{50} was 649 µg/ℓ. Na-PCP caused abnormal development of eastern oyster (Crassostrea virginica) embryos, the 48-hour EC_{50} being 40 µg/ℓ.

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Toxicity of Pentachlorophenol and Related Compounds to Early Life Stages of Selected Estuarine Animals

PATRICK W. BORTHWICK and STEVEN C. SCHIMMEL

Abstract—Newly hatched individuals of four estuarine species were exposed to pentachlorophenol (PCP), sodium pentachlorophenate (Na-PCP), or Dowicide® G (79% Na-PCP), in static toxicity tests.

The 96-hour LC_{50} values for sheepshead minnow *(Cyprinodon variegatus)* fry exposed to PCP at ages 1-day, 2-wk, 4-wk, and 6-wk were 329, 392, 240, and 223 μg/l, respectively. The 96-hr LC_{50} value for 2-wk-old fry exposed to Dowicide® G was 516 μg/l. The larvae (48-hr post hatch) of pinfish, *Lagodon rhomboides,* were particularly sensitive to Na-PCP (96-hr LC_{50}: 38 μg/l) and Dowicide® G (96-hr LC_{50}: 66 μg/l). For 24-hr-old grass shrimp *(Palaemonetes pugio)* larvae exposed to Na-PCP the 96-hr LC_{50} was 649 μg/l. Na-PCP caused abnormal development of eastern oyster *(Crassostrea virginica)* embryos, the 48-hr EC_{50} being 40 μg/l.

Introduction

Pentachlorophenol (PCP) and its water soluble sodium salt, sodium pentachlorophenate (Na-PCP),are broad spectrum biocides used in an array of pesticide formulations such as Dowicide® G (79% Na-PCP). Although there have been numerous reports on the toxicity of these compounds to freshwater organisms,

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little is known about their effects on estuarine and marine organisms. The latter is of particular interest because of the use of PCP formulations in oil and gas exploration. Pentachlorophenol and its sodium salt are used in drilling muds and well completion fluids to control bacterial growth (Robichaux, 1975), and Dowicide® G is used as a preservative in drilling fluids (Shaw, 1975). The impact of such biocides, used in land-based, coastal, and offshore oil and gas well-drilling operations, on estuarine and marine animals is not yet established. Further, we lack sufficient monitoring information, and data on the quantities used, point source disposal, and dispersal patterns of PCP formulations used in the coastal and marine environment. Land (1974) noted that microorganisms in drilling fluids are less sensitive than fish to Dowicide® G and proposed the use of formaldehyde as an alternative. Zitko (1975) suggested that PCP be eliminated as a bactericide in well-drilling operations due to the presence of highly toxic impurities (e.g. dioxins) and their acute toxicity to aquatic animals.

Because of its consistent toxic effect, sodium pentachlorophenate was selected by Davis and Hoos (1975) as an ideal reference toxicant for static freshwater salmonid bioassays: 96-hr LC_{50} values reported for juvenile rainbow trout *(Salmo gairdneri),* coho salmon *(Oncorhynchus kisutch),* and sockeye salmon *(Oncorhynchus nerka)* ranged from 37 to 130 μg/l (37 to 130 ppb).

The purpose of our study was to determine the toxicity of PCP and related compounds to sheepshead minnow *(Cyprinodon variegatus)* fry, prolarval pinfish *(Lagodon rhomboides),* grass shrimp *(Palaemonetes pugio)* larvae, and developing embryos of the eastern oyster *(Crassostrea virginica).*

Developmental stages are selected for testing because they are often more sensitive than adult stages to toxic substances. Planktonic larvae and demersal fish embryos and fry usually have inadequate mobility to avoid polluted habitats. Further, small individuals are optimal for static toxicity tests.

Experimental Animals and Bioassay Procedures

Acute 96-hr static toxicity tests were conducted following standard methods (American Public Health Association, 1976). Toxicity tests were performed using pentachlorophenol (analytical grade, Baker), sodium pentachlorophenate (analytical grade, City Chemical Company),and Dowicide® G (Dow Chemical Company). Stock solutions of PCP were made in triethylene glycol and diluted to the desired concentrations with filtered (5 μm) seawater. Solutions of Na-PCP and Dowicide® G were made by dissolving them in filtered seawater. Jars containing the exposure media and test animals were placed randomly in a temperature controlled recirculating water bath under 12 hr light: 12 hr dark conditions. The salinity and temperature at which the tests were performed varied with the species and this information is summarized in Table 1.

Sheepshead minnows were seined from tidal ditches and ponds on Santa Rosa Island, Florida, and adapted to exposure conditions for two weeks in flowing seawater which was diluted to 10‰ salinity with deionized water and heated to

30 °C. Brood stock were fed a diet of frozen adult *Artemia* and Biorell® Tropical Flake Food daily.

Sheepshead minnow fry were obtained by methods outlined by Schimmel *et al.* (1974). Adult females adapted to laboratory conditions were injected intraperitoneally with 50 I.U. of human chorionic gonadotropin to insure egg maturation. Manually stripped ova were incubated for 30 minutes in seawater with macerated testes taken from adult male fish to produce embryos, and they were tested at specified post-hatch ages (1 day, 2 wk, 4 wk, and 6 wk). Sheepshead minnow fry were fed live *Artemia* nauplii daily until their use in toxicity tests. Each bioassay consisted of five logarithmic toxicant concentrations (100 to 1000 μg/l) and appropriate controls. Sheepshead minnow fry were transferred to clean jars containing freshly prepared media, and observed every 24 hours to determine mortality or aberrant behavior.

Prolarval pinfish (48-hr post-hatch) used in the toxicity tests were obtained by using the method described by Schimmel (1977). The concentrations of Na-PCP used were: 5.6, 10.0, 18.0, 32.0, 56.0 and 100 μg/l; for Dowicide® G: 18.0, 32.0, 56.0, 100 and 180 μg/l. Pinfish larvae were not fed during the 96-hr test.

Grass shrimp larvae were obtained following the method described by Tyler-Schroeder (1976). Grass shrimp larvae (< 24 hr) were exposed to Na-PCP for 96 hours under static conditions. The larvae were fed brine shrimp daily, approximately 10 brine shrimp/larva.

Spawning was induced in the eastern oysters under laboratory conditions by an elevation of temperature to 32 °C and by the addition of 'stripped' spermatozoa to the medium. The methods of Woelke (1972) were used for the 48-hr exposure of oyster larvae to Na-PCP. The concentrations of Na-PCP tested were: 3.2, 10.0, 32.0 and 100 μg/l. After the 48-hr exposure the embryos were examined for abnormalities in development to the straight-hinged stage. Larvae were considered normal if their shells were fully formed.

Probit analysis (Finney, 1971) was used to determine the 96-hr LC_{50} value, using death as the quantal response for sheepshead minnows, pinfish and grass shrimp. Similarly, the 48-hr EC_{50} value was determined for eastern oyster embryos using abnormal development of experimental embryos as the quantal response.

Toxicity of PCP and Related Compounds

The acute toxicity of PCP and related compounds to laboratory-reared sheepshead minnow fry at various ages is shown in Table 1. The sheepshead minnow fry of 4- and 6-weeks age appeared to be more sensitive to PCP than did the day-old and 2-week-old individuals. Death of sheepshead minnow fry generally occurred during the first 24 hours of exposure. Death was characterized by laterally extended opercula and reddened areas around the pectoral fins. Aberrant behavior included hyperventilation and sluggish response to mechanical stimuli.

For 2-wk-old fry exposed to Dowicide® G, the 96-hr LC_{50} value was 516 (374-887) μg/l, while a 96-hr LC_{50} of 392 (307-489) μg/l was obtained with PCP. These

TABLE 1. Toxicity of PCP, Na-PCP and Dowicide® G to Early Life Stages of Estuarine Animals

Species	Age and stage	Chemical	96-hr LC_{50} µg/l (ppb)	95% C.I.	Temp. °C	Salinity ‰
Cyprinodon variegatus (sheepshead minnow)	1-day fry	PCP	329[a]	(305-360)	30	10
	2-wk fry	PCP	392	(307-489)	30	10
		Dowicide® G	516	(374-887)	30	24
	4-wk fry	PCP	240	(192-284)	30	10
	6-wk fry	PCP	223	(163-291)	30	10
Lagodon rhomboides (pinfish)	48-hr prolarvae	Na-PCP	38	(26-57)	20	26
		Dowicide® G	66	(48-94)	20	26
Palaemonetes pugio (grass shrimp)	24-hr larvae	Na-PCP	649	(494-915)	25	24
Crassostrea virginica (eastern oyster)	developing embryos	Na-PCP	40[b]	(36-44)	25	17

[a]The values given are based on nominal concentrations.
[b]48-hr EC_{50} value for abnormal embryonic development.

differences may have been due to the fact that only 79% of the former formulation is Na-PCP while the latter is an analytical grade PCP with less contamination.

Parrish *et al.* (1977) reported the 96-hr LC_{50} and 95% confidence limits for juvenile (10-20 mm) sheepshead minnows exposed to PCP in flowing seawater to be 442 (308-635) μg/l. These values are somewhat close to the LC_{50} values obtained in the present investigation for 1-day-old and 2-week-old sheepshead minnow fry exposed to PCP for 96 hours.

Pinfish prolarvae (48-hr-old) were particularly sensitive to Na-PCP and Dowicide® G, the 96-hr LC_{50} values being 38 (26-57) and 66 (48-94) μg/l, respectively.

The 96-hr LC_{50} for laboratory-reared grass shrimp larvae exposed to Na-PCP was 649 (494-915) μg/l. Eastern oyster embryos exposed for 48 hours to Na-PCP exhibited abnormal development, the 48-hr EC_{50} being 40 (36-44) μg/l. Woelke (1972) exposed Pacific oyster *(Crassostrea gigas)* larvae to Na-PCP. Based on his tabular data on abnormal embryonic development the 48-hr EC_{50} appears to be 48 μg/l.

In conclusion, our study indicates that PCP and related compounds are toxic to developmental stages of several estuarine animals. Among the individuals tested the prolarval pinfish are extremely sensitive to Na-PCP (LC_{50}: 38 μg/l). At a similar concentration, Na-PCP caused abnormal development in eastern oysters. Na-PCP and PCP appear to be more toxic than Dowicide® G to prolarval pinfish and sheepshead minnow fry.

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